

Fig. 1

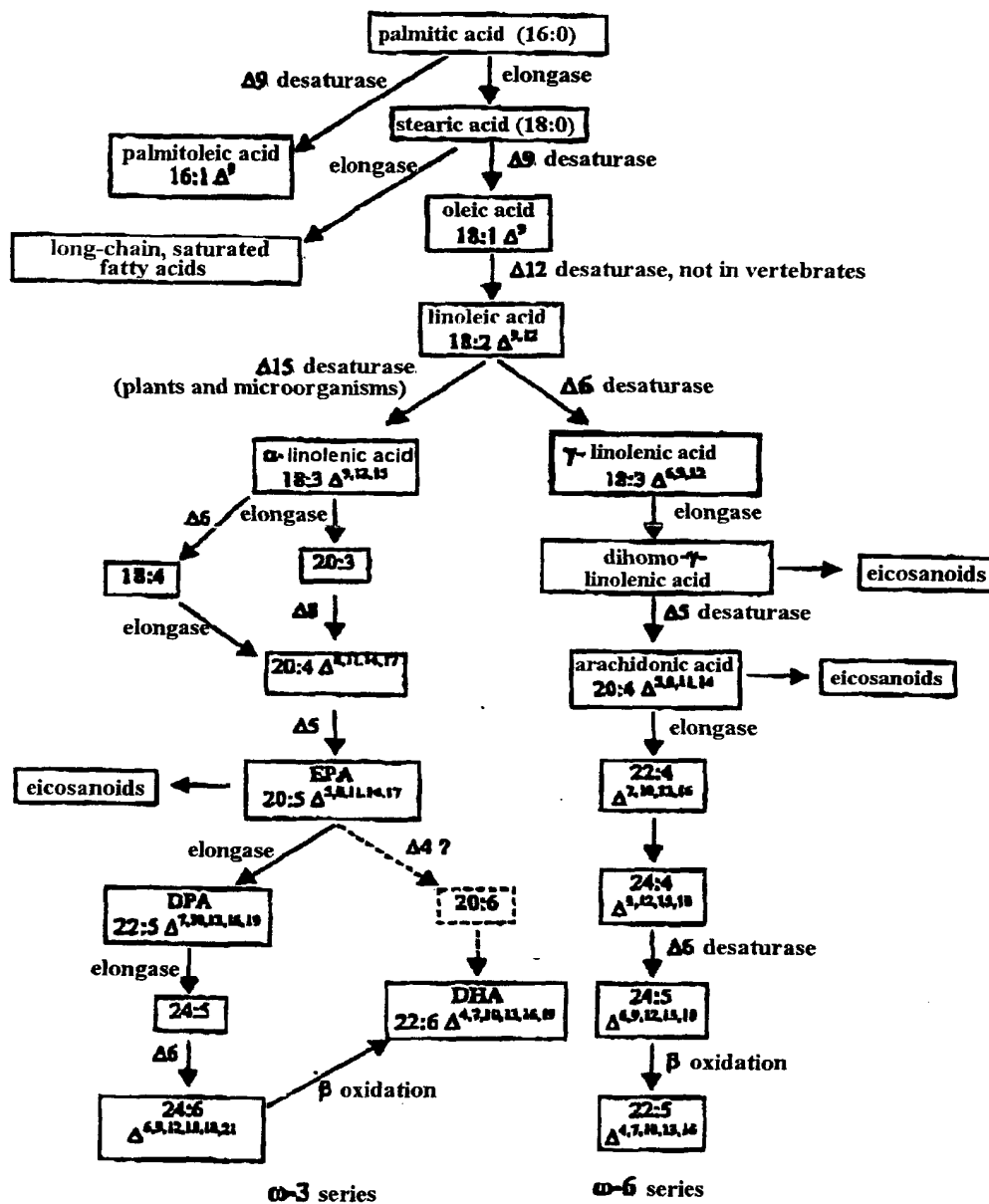


Fig. 2

BLAST 2.0.0 (Jan-05-1999)

Reference: Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", *Nucleic Acids Res.* 25:3389-3402.

Query= T. thermophila, delta-6-Desaturase (352 letters)

Database: /LION/data/db/fast/nrdb

387,705 sequences; 119,829,732 total letters

10

# Sequences producing significant alignments:

		Score	E
		(bits)	Value
15	trembl AF078796 AF078796_1 gene: "des-5"; product: "delta 5 fat...	79	4e-14
	trembl AF031477 AF031477_1 product: "delta6-fatty-acid-desaturase...	79	4e-14
	trembl X61122 CE713F2_1 gene: "T13F2.1"; Caenorhabditis elegans...	78	1e-13
20	trembl Z70271 CEW08D2_2 gene: "W08D2.4"; Caenorhabditis elegans...	78	1e-13
	trembl AF035096 AF035096_1 product: "desaturase/cytochrome b3 p...	70	3e-11
	trembl AJ222980 D7AJ2980_1 gene: "des6"; product: "delta6-acyl-...	69	6e-11
	trembl U79010 BOU79010_1 product: "delta 6 desaturase"; Boreo...	67	2e-10
	trembl NC003397 NC003397_14 gene: "T3F17.14"; product: "putativ...	67	2e-10
25	trembl AF007561 AF007561_1 product: "delta 6-desaturase"; Bora...	66	4e-10
	tremblnew AF126799 AF126799_1 product: "delta-6 fatty acid desat...	64	2e-09
	tremblnew AF126798 AF126798_1 product: "delta-6 fatty acid desat...	63	3e-09

2A/M0

	tramb1 AT031194 AT031194_1 gene: "B278", product: "B278", tclt...	62	6e-09
	tramb1new AB021980 AB021980_1 product: "delta-5 fatty acid desa...	62	6e-09
30	tramb1new AL078610 SC235_12 gene: "SC235.42c", product: "putati...	62	8e-09
	tramb1 AJ224160 BNJ4160_1 gene: "ald1", product: "delta-8 sphi...	60	2e-08
	tramb1 AC004770 AC004770_2 product: "BC269730_2", Homo sapiens...	60	3e-08
	tramb1 AJ224161 ATJ4161_1 gene: "ald1", product: "delta-8 sphi...	59	6e-08
	tramb1new AL050110 HSH800210_1 gene: "HSH800210", product: "...	57	2e-07
35	tramb1 AB022097 AB022097_1 product: "delta 5 fatty acid desatur...	57	2e-07
	tramb1 X07143 HACTT838N_1 product: "cytochrome b5 containing fa...	50	2e-05
	tramb1 Y08460 HMH08_1 gene: "Hden", product: "Hden protein", ...	50	3e-05
	tramb1 AT001394 AT001394_1 product: "fatty acid desaturase/cyto...	46	4e-04
	tramb1 AF002669 HSA72669_1 product: "HLD", Homo sapiens putati...	46	5e-04
40	swiss Q08871 LALCD_STRT3 LINOLEOYL-CoA DESATURASE [EC 1.14.99.25...	43	0.003

Continuation of Fig. 2

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**Fig 3A**

[illegible]

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**Fig. 3B**

**Fig. 3B**

>trebbj|AV031477|AV031477 1 product: "delta6-fatty-acid-desaturase";  
ConservedAbditi elegans delta6-fatty-acid-desaturase wds, complete cds. //rplnrv031477|3089520  
product: "delta6-fatty-acid-desaturase"; ConservedAbditi elegans delta6-fatty-acid-desaturase  
wds, complete cds. length = 463

Score = 79.2 bits (1192), Expect = 4e-10  
Identical = 100/390 (25%), Positives = 132/390 (33%), Gaps = 95/390 (24%)

Query: 41 KPGGKLVNLPVETQGVTVPTGLS---RPALEKIVLSTPPTQALQ--RTTSEHRL--- 93  
KPPGS D Y F H S QM L K C E S + M  
Subject: 28 KRPSSAVTEQT---NMGRATVPATVDSIPIVATVLMKKKDKDSTLAKLKKLQLK 84

Query: 94 -----FSLIKLLY-LPTWPTLIGLVTITTTTLPTTCULPD 130  
F L+EL L + B + ++F ++  
Subject: 85 VDIWNVNVPVAGKKNVSTFLAQVDCVZANVSTLTITLTIKISLVAFTLQ 146

Query: 131 R-WTF-SIPVLVLAIVLGGKSHSR---WPLAKLVAYAPLCCSTNNHWNR 183  
WT S LL L GM+ B B + P+ GL+ GTB W N  
Subject: 145 YLCITTTCTCLALAAQQCGMLVREICVDPTTETLDTTILSLTPCFLQGCPFWNNWR 204

Query: 184 ENKRNHTVMTLKQSI-----QDVVKWPTPTLP 213  
EN RN 7N I D FI CM Y P L  
Subject: 205 ENRVNHTVINDSDGTDLAVALPAPKSCSTQAFPRALIAVYFGSTTACLPKLR 266

Query: 216 LAWEKLGGTLAGVVE-----FEGILVNTLLPWPTVI-----VILS 251  
W B+ + E +E + EN +F O F + 1+E  
Subject: 265 PSTSGQSVPTTESQGVTVVDMATPCSTVETERNVPTTGILLPTTWELNAVPTLS 324

Query: 252 ELIACITNSTLVCHRRHEWF---ENVIITATPCDIAASVATAVDFIALINCKMDIO 309  
++ G A ++ HB + B+ AL F QI +RM L GS+ IQ  
Subject: 325 CGSGALLAVTVPTPVNSVTPVPSVSLVSLVATLALQILITCTDTRPTPTIQNL-NCSLATD 303  
Query: 318 TDEHTPTGRTTFLPFAVITIAKSNL 339  
ENK TP +P L + B R+ NL  
Subject: 384 IENHPTTHSPCHCALCVTAIVALKNGIL 413

**Fig. 3C**

>trambi:UT95010190073810 il prodotto: "delta 6 desaturase"; Storage officialita delta 6 desaturase  
se non, complete cds. 77,9617959101026203 product: "delta 6 desaturase"; Storage officialita  
delta 6 desaturase mRNA, complete cds. length = 418

Score = 67.1 bits (151), Expect = 2e-10  
Identities = 100/416 (24%), Positives = 196/416 (37%), Gaps = 105/416 (24%)

Query: 6 TUES(V)JANFLLAJETUTU IYUOTVSTAYALSSBNCCLWFLAFTIUSOONLZETVT 69  
7 W+ +W + + TO +++ E MDG L O++P F  
Subject: 10 TUELOKNNH--CGLTITOCNATVUNVED--SPSFFFAJLAS--DETVALVA 62

Query: 68    LUNEDALSTJERNEN    19401-----AFSTJERNELISTEN 112  
           H    K L P Q    ++    +R    +    +    +    +    +    +  
 Object: 63    19401LUNEDALSTJERNEN    19401LUNEDALSTJERNEN 121

**Subject: 63 REPASTWELDOR-PROTIZLOTVVIVVIDTROZLVNTPBEMLYOUEKHNINUTATZCFIA 121**

[illegible]

Query: 169 APLC-CALIFORNIA-CHS-RTM-INDICION  
A C C S W E S H+ W+ D B+Q+

July: 203 ----- 240

Query: 203 -----SYNOPSIS OF THE PROCEEDINGS-----PAGE 171-172-240  
 Y + W + 1/4 + 8 42 C + W L  
 Subject: 240 DOLANVS DONTTPPINC AAKUMTOSIMIALPCHVS YANCLACCLYBTPPL 299

Query: 241 -----FACIITVIGSLAIF-----FASIAVCHHCHCZTALU7L77799 205  
           + + VI A + B         F9-84 VC + F9-4 Y +  
 Object: 300 VBCLNWCEBURYIASLVATGQOWUMIMUBISSTVCSPCHNINFEK0-F0211018 350

emory: 206 LANSSTATEDISFELLSINGCOTY78MUTTTTJUSPTLAWVIAKACOMTEL 339  
++ IN CO-D-O COM FP+P LE + E KE NL  
bjct: 339 CPMON-TH-----CELCTIVEMLTFFRFRSICATGAL 403

Subject: 339 CPMW-77-----RELATIONSHIP BETWEEN VIOLENCE AND

**DE  
FLY**

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>idna|048971|L2CD BYTES LINGUA-CON DEASTURUS (IC 1.16.99.25)  
 (0007416)-DEASTURUS, //trembl|12121|80003 1 product: "delta-6 deasturase", *Synschochyatla*  
 sp. delta-6 deasturase gene, complete cds. //trembl|04941|185014 112 genes: "dast" products:  
 "delta-6 deasturase", *Synschochyatla* sp. PC6803 complete genome, 16/27, 1391336-2137238.  
 //nslmaly|131517|83157 delta(6)-deasturase - *Synschochyatla* sp. //tw|050514|105389 genes:  
 "dast", product: "delta-6 deasturase", *Synschochyatla* sp. PC6803 complete genome, 16/27,  
 1391336-2137238. //cp|14121|16363 product: "delta-6 deasturase", *Synschochyatla* sp. delta-6  
 deasturase gene, complete cds. length = 359

Score = 43.4 bits (100), Expect = 0.003  
 Identities = 63/200 (21%), Positives = 101/200 (100), Gaps = 61/200 (21%)

[illegible]

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Query: 176 *****-----*****  
      E+ W +MS EX +TS + O +I B + P . F W L +  
Object: 118 *****
```

diary: 218 YRZGKQZAL-----HWLLRGVTVIVL9-----LTJGFTHSIL96 219  
           Y+         E + F         +  
 pict: 175 TRLWLVZMKTUOITDZPZYKSLAR/GUJGVNCAIAGTIPRYAG 210

[illegible]

Project: 201 LINECQVOTHSB77FQJPTTRAFWYDANRLEPOMKXNOCZYTH 348  
GSA + O EN EV I P+ II + +++ ++ # P+

Project: 293 MFCOUMGVULFRHICISIPOLMNOZCVLWVAVTTH 312



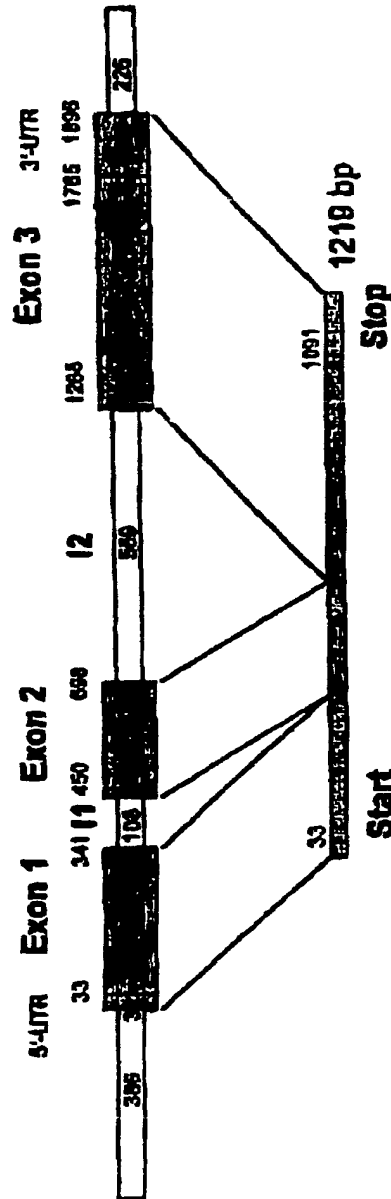
Fig. 4

5	M. alpina	1	-----HAAAYVNTTAAVYVNA-----GDAEAP-----	1	-----D-----
	C. elegans	1	-----KDE-----HES-----	1	-----LA-S-----
	B. officinalis	1	-----NARQIKVYTSQ-----PCT-----	1	-----K-D-----
	M. musculus	1	-----NWKGGDGGSTGCAAPPTTNS-----LAT-----	1	-----EO-R-----
	T. thermophila	1	-----HCVNTQD-----PELLN-----	1	-----KENV-----
10	M. alpina	54	-----SVV-----	54	-----A-----
	C. elegans	54	-----KSKKANG-----	54	-----KDDPKINGDDV-----
	B. officinalis	45	-----STF-----	45	-----V-----
	M. musculus	57	-----BRV-----	57	-----P-----
	T. thermophila	46	-----LAF-----	46	-----P-----
15	M. alpina	100	-----FAN-----	100	-----FAN-----
	C. elegans	91	-----NMGTFNLS-----	91	-----NMGTFNLS-----
	B. officinalis	91	-----K-----	91	-----K-----
	M. musculus	105	-----KSSQIT-----	105	-----KSSQIT-----
	T. thermophila	92	-----K-----	92	-----K-----
20	M. alpina	146	-----QVATLANS-----	146	-----QVATLANS-----
	C. elegans	146	-----LQTHYV-----	146	-----LQTHYV-----
	B. officinalis	133	-----PCEGVVH-----	133	-----PCEGVVH-----
	M. musculus	155	-----TGMHPT-----	155	-----TGMHPT-----
	T. thermophila	126	-----LQVQW-----	126	-----LQVQW-----
25	M. alpina	199	-----KQV-----	199	-----KQV-----
	C. elegans	205	-----SGG-----	205	-----SGG-----
	B. officinalis	186	-----QIC-----	186	-----QIC-----
	M. musculus	207	-----A-----	207	-----A-----
	T. thermophila	174	-----A-----	174	-----A-----

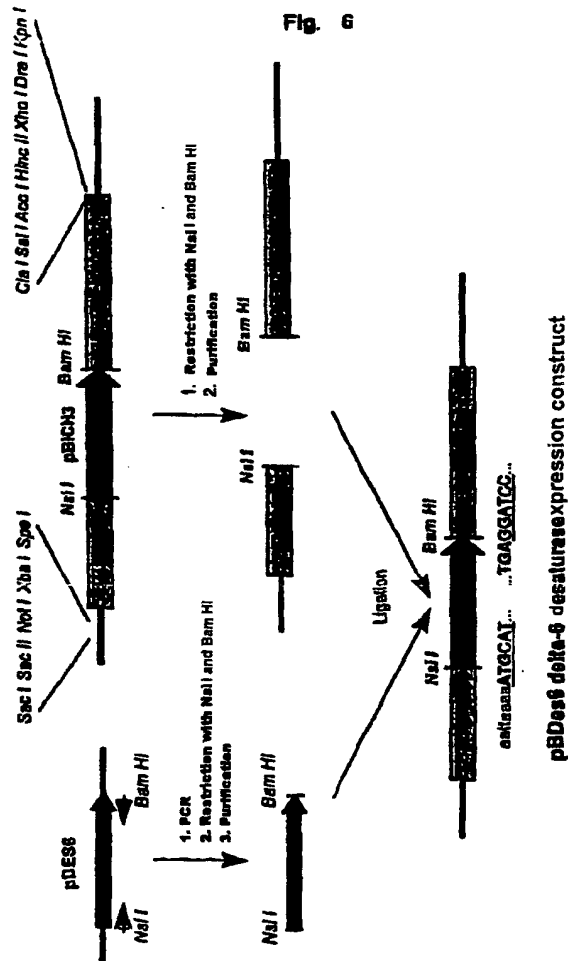
#### Continuation of Fig. 4

Fig. 5

# Structure of the $\Delta$ -6-desaturase gene



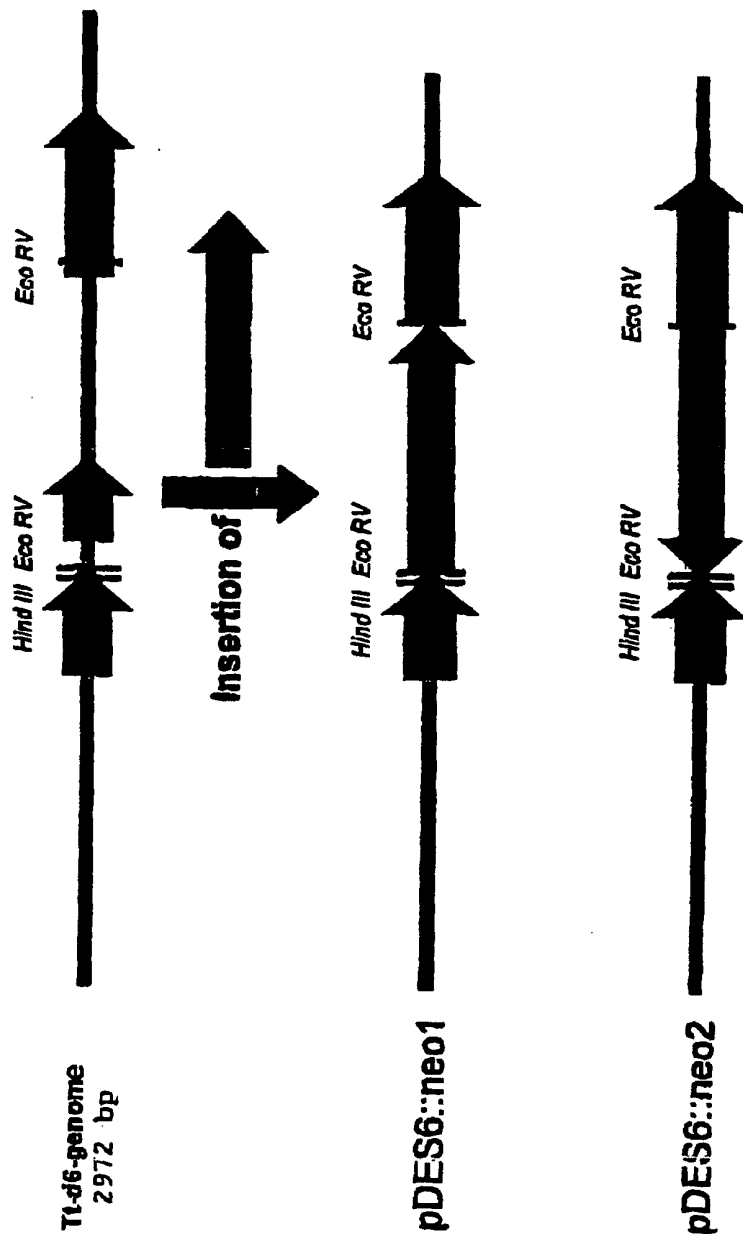
# Preparation of the pDES6 expression construct



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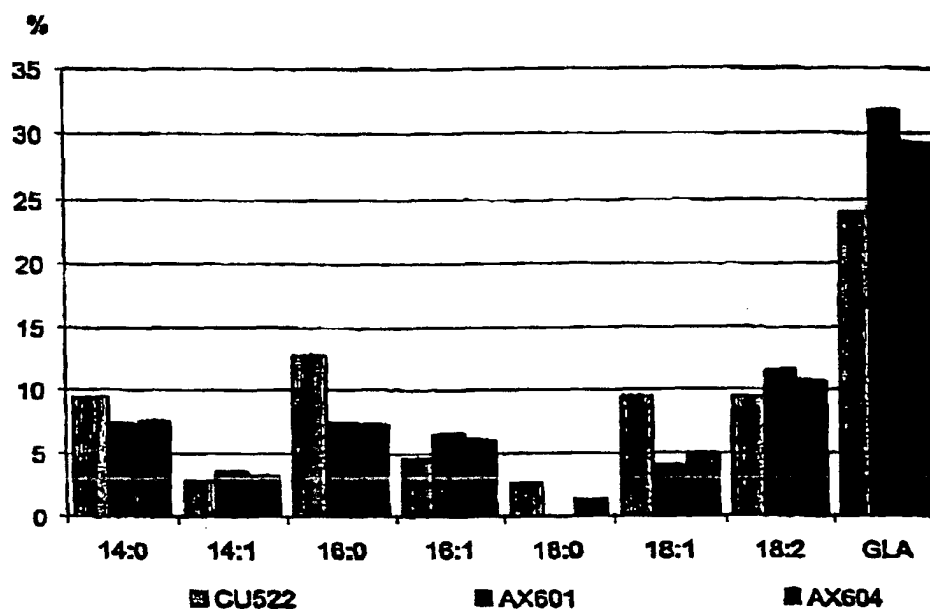
Fig. 7

# **Delta-6-desaturase knockout construct**



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Fig. 8



Comparison of the fatty acid spectrum (main fatty acids) of the Tetrahymena pBDES6 transformants (AX601 and AX604) with that of the Tetrahymena wild-type strain (CU522) after 50 h of culture. The figure gives the percentage content of the main fatty acids in the total fatty acid spectrum